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Stipitate hydnums of the southern United States 1: *Phellodon mississippiensis* sp. nov.

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Abstract—During a general survey of fleshy fungi from Mississippi, an undescribed *Phellodon* species was observed in the Tombigbee National Forest at the same location over a two-year period. No other studies of the stipitate hydnums are available for the midsouthern states that directly compare with the new species. Molecular sequence data of *P. mississippiensis* were distinct from other *Phellodon* spp. of the southeastern United States. The highly fused (concrescent) basidiomata of *P. mississippiensis* are found under mixed hardwoods and associated pines. The light tan to light rust (orange) or salmon color on the marginal spines and along the margin of the small (≤5 cm broad) stout spongy tomentose basidiomata makes this species distinct. The presence of rare clamp connections was observed in the trama hyphae. However, identification of *P. mississippiensis* can be complicated by its concrescent nature and the indeterminate growth forms caused by environmental conditions. Morphological characters and results from the phylogenetic analysis are included in the species description.

Key words—white spored, mycorrhizal, early summer, fenugreek odor

Introduction

The stipitate hydnums in the southeastern United States have been studied extensively either directly or in larger monographs (Banker 1906, 1913a,b,c; Coker 1919, 1926, 1927, 1939, 1942; Jones 1935; Coker & Beers 1951; Baird 1986a,b, 1987; Baird & Kahn 1986, 1987). However, no specific studies of the group have been conducted for the mid-south, including Mississippi. David Lewis (Gulf States Mycological Society, pers. comm.) reports that many species of fleshy fungi have been identified and new species recorded in numerous published research papers from the state. Lewis stated that F.S. Earle & L.M. Underwood spent time along the Gulf Coast in the Ocean Springs, MS, area, collecting primarily *Amanita* and *Boletus* spp. Many of these species were

sent to W.A. Murrill, who published many as new. More recently W.G. Cibula extensively documented fleshy fungi for Mississippi (Cibula 1979, Cibula & Weber 1996), sending most collections sent to the Field Museum, Chicago, IL. Finally, of the three stipitate hydnums cited in Dukes' (2000) book on Mississippi fleshy fungi, two were correctly identified to genus as *Hydnellum* and another listed as *Phellodon* was incorrectly identified and was actually a wood decay fungus.

A study of the stipitate hydnums from southern Appalachian Mountains represents the most recent monograph on stipitate hydnums from the southern region (Baird 1986a). During that research, a thorough type study was also conducted with worldwide implications (Baird 1986b). More recently revision and phylogenetic analyses (unpublished data) of stipitate hydnums have shown that nine *Phellodon* spp. from Great Smoky Mountains and the surrounding southern Appalachian Mountains are distinct.

During a general survey of macrofungi in northern Mississippi, an unknown *Phellodon* sp. (Fig. 1) was collected from the same location during 2009 and 2010. The collections were found under mixed hardwood with conifers near the site. Morphological comparisons with all other known *Phellodon* spp. from southeastern United States indicated that none of the general herbarium collections or from previous type studies were similar (Baird 1986b). Furthermore, a BLAST search of molecular sequences from other *Phellodon* spp. from the southeastern United States and GenBank revealed no matches, supporting the species as undescribed. We present here a detailed description of the new species and its phylogeny (Fig. 3).

Materials & methods

In 2009 and 2010, collections were obtained from the North Trail sect. D of Noxubee Crest Mountain Bike Trail System of Tombigbee District of Tombigbee National Forest. Survey of associated tree species was conducted within a 30 m radius of the basidiomata.

All macro- and microscopic data were analyzed according to Baird & Kahn (1986). Colors were determined with Kornerup & Wanscher (1978). Digital images were taken of slide mounts of basidiospores and basidia using a Jenoptik ProgRes 3.3 megapixel digital camera mounted on a Olympus BX50 compound microscope. Basidiospore, basidium, and sterigma sizes were measured from these images using image-analysis software (IMT i-Solution Lite), and 20–40 measurements were made for each of three variables per collection. Scanning electron microscope (SEM) photographs were made of dried specimen material mounted on aluminum stubs with double-sided sticky carbon tape, coated with 15 nm platinum, and examined with a JEOL JSM 6500F SEM. The accelerating voltage was 5 KV and the images were digitally recorded.

Molecular examination

The nrITS DNA region from the new species was sequenced for phylogenetic comparisons with other *Phellodon* species. Genomic DNA was extracted from dried

basidiomata tissue using DNeasy, Plant Mini Kit by Qiagen (Valencia, CA) following procedures discussed in the brochure (08/2000) instructions. The ITS primers ITS1F (Gardes & Bruns 1993) and ITS 4 (White et al. 1990) were used to amplify the internal transcribed spacer region ITS 1-5.8S-ITS 2. Amplification reaction for ITS used procedures previously described (Lickey et al. 2007). The PCR products were purified with Qiagen PCR columns (Qiagen, Chatsworth, CA) and sequences by Eurofins-MWG/Operon (Huntsville, AL). Resulting electropherograms were viewed and edited using DNAstar of Lasergene (Madison, WI), and multiple sequences per taxon were collapsed due to lack of variation. When electropherograms were unreadable due to multiple sequences or insertion-deletions that could not be resolved, PCR products were cloned using pGEM-T vector system and SM 109 competent cells following manufacturers protocol (Promega). Sequences generated include *Phellodon* sp. 1 (TENN65891 = JN135199), *P. alboniger* (Peck) Banker (TENN65868 = JN135206), *P. brunneoolivaceus* (Coker & Beers) R.E. Baird (TENN65882, JN135201), *P. confluens* (Pers.) Pouzar (TENN65874= JN135198), *P. ellisianus* Banker (TENN65884= JN135204), *P. fuligineoalbus* (Fr.) Baird (in press) (TENN65893= JN135196), *P. fibulatus* K.A. Harrison (TENN65865= JN135205), *P. melaleucus* (Sw. ex Fr.) P. Karst. (TENN65896= JN135197), *P. niger* (Fr.) P. Karst. (TENN65876= JN135202), *P. putidus* (G.F. Atk.) Banker (TENN65883= JN135200), and *P. tomentosus* (Fr.) Banker (TENN65889= JN135203). Sequences of *Sarcodon atroviridis* (Morgan) Banker (TENN65871= JN135190), *S. imbricatus* (L.) P. Karst. (TENN65890= JN135194), and *S. scabripes* (Peck) Banker (TENN65894= JN135191) were designated as outgroup. All sequences were subjected to GenBank program BLAST to detect similar sequences, resulting in the inclusion of six additional sequences in the data set: *Phellodon* sp. 352-363 (AB509722), *P. nothofagi* McNabb PDD89898 (GU222318), *P. nothofagi* PDD89880 (GU222316), *P. atratus* K.A. Harrison (HQ650766, FN185793), and *P. sinclairii* (Berk.) G. Cunn. (GU222291). Sequences of the new species were submitted to GenBank and accession numbers JN247563 (MS-1) and JN247564 (MS-3) were designated.

The sequences were aligned using the web version of MAFFT (Katoh et al. 2005) with the G-INS-i option, which is provided by the CBRC (http://mafft.cbrc.jp/alignment/server/). The resulting alignment of 1,105 characters was used directly in jModeltest (Posada 2008) to determine TrN+G as the best fitting model of molecular evolution under the AICc. A Bayesian phylogenetic analysis was performed on the data set using MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003). The three *Sarcodon* sequences were designated as outgroup. Using a GTR+G model the MrBayes analysis was conducted for 1 million generations, after which the split standard deviation reached 0.006. A burn-in of 2,500 trees was used prior to determining the posterior probability of the trees with the highest likelihood. The consensus tree is reported with posterior probability indicated as support for clades.

Taxonomy

Phellodon mississippiensis R.E. Baird, L.E. Wallace & G. Baker **sp. nov.** FIGS 1–2

INDEX FUNGORUM IF550077

Differs from *Phellodon putidus* and *Phellodon tomentosus* by its salmon colored spines, very stout fused pilei or stipes, and presence of clamp connections.

Type: United States of America. Mississippi, Noxubee County, Tombigbee National Forest, North Trail sect. D of Noxubee Crest Mountain Bike Trail System, (35°44.350′N 83°25.415′E), June 15, 2010, P. Scott, MS-3 (**holotype,** TENN65879; GenBank, JN247564).

Etymology: The epithet refers to the State in which the type was collected.

Basidiomata single, usually highly concrescent, ≤5.0 cm broad, stout, convex to plane or subdepressed or rarely colliculose; margin blunt in immature specimens to thin or irregular in age; spongy-tomentose becoming matted or appressed at disc, smooth to pitted or irregular at disc in aged tomentum, rarely radially rugulose, white (5A1) to orange white (5A2) or light orange (5A4) nearest margin, light brown (6D5), cinnamon (6D6) to burnt umber (6F6) or dark brown (6F7) at disc; underside of pileus margin and immature spines white (5A1) to pale orange (5A3) or light orange (5A5); context ≤3.0–4.0 mm thick nearest stipe, evidence of zones, concolorous with pileus. Stipes ≤1.5 × 2.0 cm concrescent or <1.0 × 1.0 cm single, central to eccentric, flattened or irregular on a side, tomentose becoming matted in age, bruised surface irregular, cylindrical to subattenuate above, at lower third entire base often fused forming a mycelial mass, lower portion of mycelial mass buried forming a thick network in the duff layer, white (5A1) nearest base to pale orange (5A3) or dark brown (6F6); context ≤1.5 cm thick, not obviously duplex, azonate, concolorous with pileus context. Spines up to 2.0 mm long, subcrowded, subdecurrent, white (5A1) to light orange (5A5) nearest margin. *Taste* none; *odor* moderate to strong fenugreek increasing upon drying. Chemical reaction in KOH light to dark brown on pileus context tissues over short period of time.

Pileus trama hyphae ≤5.0 µm diam, uninflated, generally interwoven in subsurface layer, parallel below, unclamped; rare occurring gloeophorous-like hyphae ≤7.0 µm diam, cylindrical to highly irregular-shaped. Stipe hyphae ≤4.5 µm diam, uninflated, interwoven in subsurface layer to parallel below, unclamped, rarely observed gloeophorous-like hyphae ≤9.0 µm diam, when present. Spine trama hyphae subparallel to parallel, ≤4.0 µm diam, rare clamp connections in subhymenial hyphae. Basidiospores 16.0–22.0 × 5.0–6.0 µm (mean = 17.9 ± 1.7 × 5.8 ± 0.5 µm), subglobose to globose, hyaline; ornamentation echinulate, spinules not prominent; hilar appendage oblique. Basidia 16.0–22.0 × 5.0–6.0 µm (mean = 17.9 ± 1.7 × 5.8 ± 0.5 µm), clavate, unclamped, 4-spored; sterigmata ≤4.0 µm long (mean = 3.6 ± 0.39 µm). Clamp connections absent in pileus and stipe contact tissues, rare in subhymenial hyphae of the spines.

Additional specimen examined: **UNITED STATES of AMERICA**. Mississippi, Noxubee County., Tombigbee National Forest, North Trail sect. D of Noxubee Crest Mountain Bike Trail System, (35°44.350′N 83°25.415′E), May 28, 2010, P. Scott, MS-1 (TENN65879; GenBank JN247563).

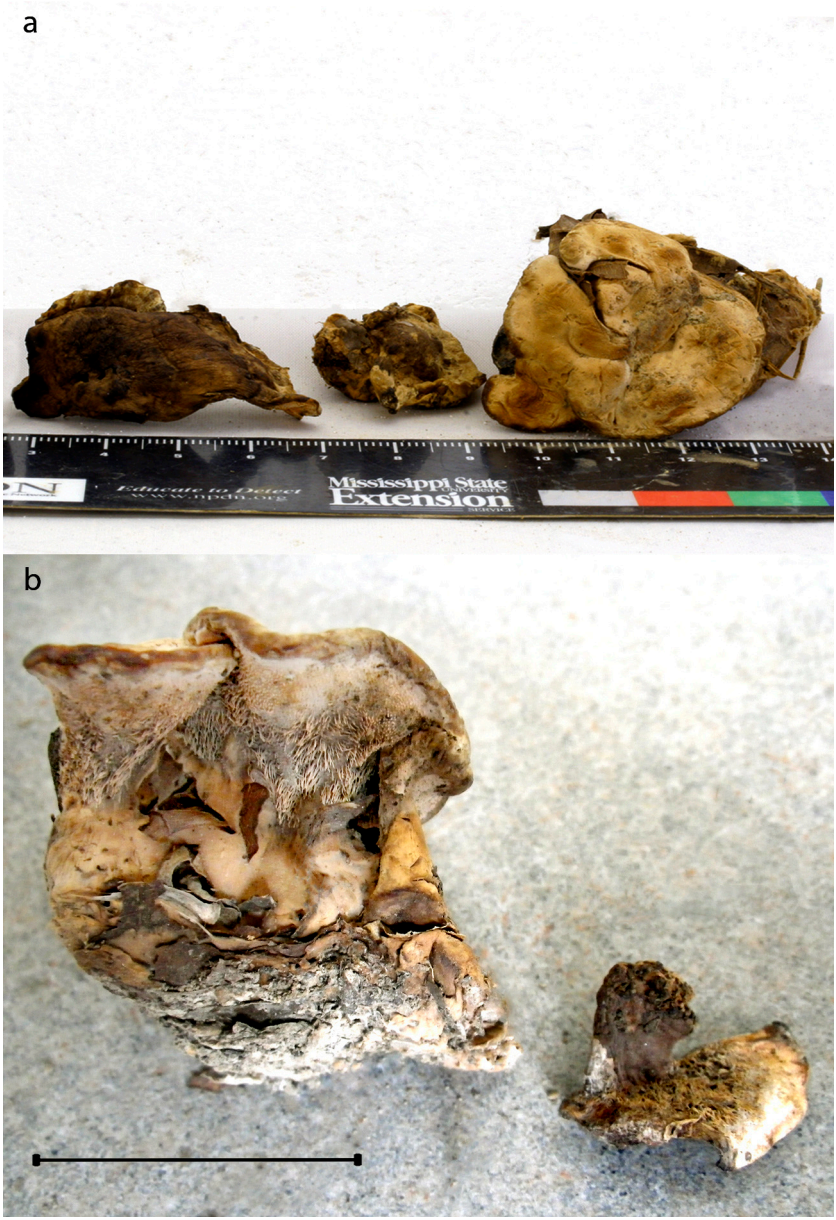

Fig. 1. *Phellodon mississippiensis.* a: Basidiomata showing upper portion of basidiomata; b: Basidiomata side view of fused pilei and stipes with thickened mycelial base. Scale bars = 5.0 cm.

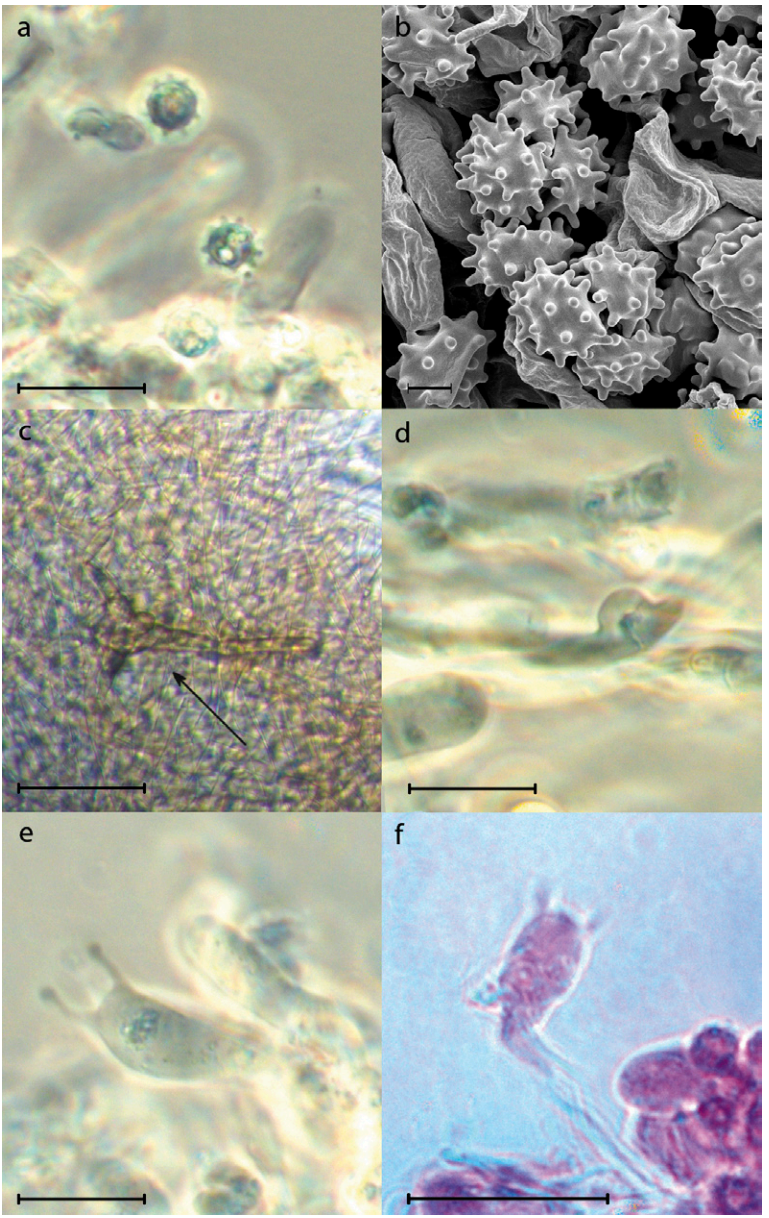

Fig. 2. *Phellodon mississippiensis*. a: Basidiospore with echinulate ornamentation; b: Scanning electron microscope (SEM) micrograph of basidiospores; c: Gloeophorous-like hyphae present in pileus and stipe; d: Clamp connection in the subhymenial hyphae; e–f: Basidia and sterigmata. Scale bar = 10 μm.

Habitat: The type location contained the following tree species (percent densities) within 17.4 m of the collections: *Pinus taeda* L. (20% = 5 trees), *Carya glabra* (Mill.) Sweet (16%), *Acer rubrum* L. (16%), *Fraxinus americana* L. (12%), *Cornus florida* L. (8%), *Oxydendrum arboretum* (L.) DC. (8%), *Ostrya virginiana* (Mill.) K. Koch (1%), *Quercus velutina* Lam. (1%), *Q. rubra* L. (1%), and *Nyssa sylvatica* Marshall (1%). The site (6.0% slope), which was 3.0 m from a small intermittent stream, also contained 30 year-old pines planted >25.0 m from the collections that were under the hardwoods.

Discussion

The phylogenetic analysis strongly supports *P. mississippiensis* as a member of the *Phellodon* clade, which is distinct from the outgroup *Sarcodon* species (100% PB; Fig. 3). The two *P. mississippiensis* samples are identical and unique within *Phellodon*, further supporting its separation as a new species. Within *Phellodon* however, the evolutionary relationship of *P. mississippiensis* with other species is unclear. Current phylogenetic analyses clearly place *P. mississippiensis* within *Phellodon* but are unable to resolve most sister species relationships within the genus (Fig. 3).

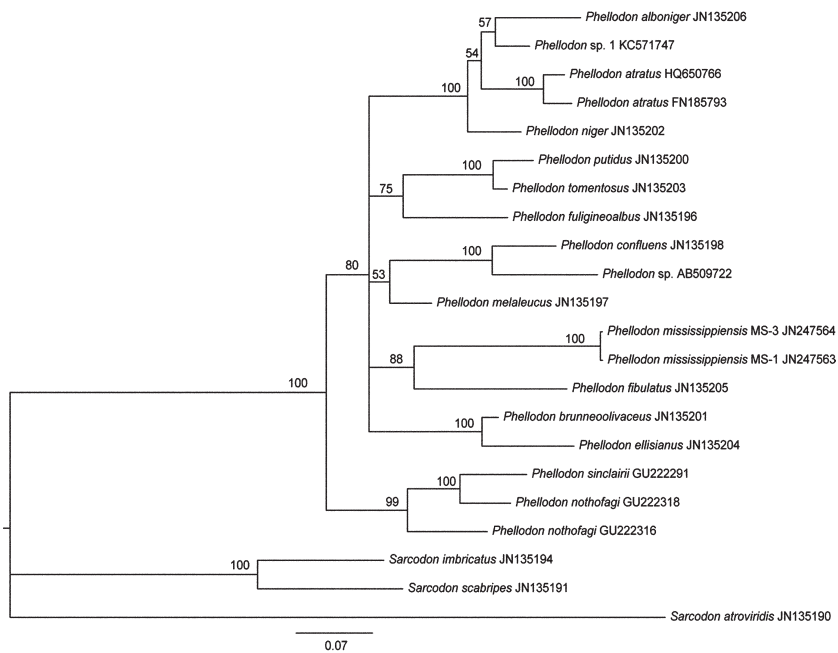

Fig. 3. Bayesian consensus phylogeny resulting from analysis of nrITS sequences. Values on branches are posterior probabilities and indicate support for nodes.

Molecular sequence data and morphological differences separate this species from all other *Phellodon* spp. known to occur in the southeastern United States, including Florida (Baird 1986a,b; Baird & Kahn 1986). Morphologically the basidiomata may be confused with several species based on indeterminate growth forms and concrescent nature, in particular, *P. fibulatus*, *P. confluens*, *P. tomentosus*, *P. radicatus* R.E. Baird and *P. putidus*. *Phellodon putidus* has marginal colors overlapping those of *P. mississippiensis* but is much larger (≤11 cm broad) and lacks the thickened fused stipe bases that can occur in *P. mississippiensis*. Basidiomata of *P. fibulatus* (with abundant clamp connections) and *P. tomentosus* (with clamp connections scattered randomly on stipe hyphae) are much thinner than *P. mississippiensis*. Unlike *P. mississippiensis*, *P. tomentosus* occurs only under conifers. *Phellodon confluens* has very thin basidiomata and lacks the light orange or salmon colors seen on the margin of *P. mississippiensis*. *Phellodon radicatus*, reported from Florida, has a blackish colored context and radicating stipe bases not present in *P. mississippiensis,* which is instead characterized by the thick mycelial mass formed from fused stipes and buried in the humus layer. Also, basidiospores of *P. radicatus* are 1.0 μm larger than in the new species.

Previous studies in Florida and surveys in the midsouth and along the Gulf Coast lead us to expect that new stipitate hydnum species will continue to be identified during surveys of this region.

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Literature Cited

Baird RE. 1986a. Studies of the stipitate hydnums from the Southern Appalachian Mountains – genera: *Bankera, Hydnellum, Phellodon, Sarcodon.* Bibliotheca Mycologica104. 156 p.

Baird RE. 1986b. Type studies of North American and other related taxa of stipitate hydnums: genera: *Bankera, Hydnellum, Phellodon, Sarcodon.* Bibliotheca Mycologica103. 89 p.

Baird RE. 1987. Chemical constituents of the stipitate hydnums from the southern Appalachian Mountains. Mycotaxon 28: 61–70.

Baird RE, Khan SR. 1986. The stipitate hydnums (*Thelephoraceae*) of Florida. Brittonia 38: 171–184.

Baird RE, Khan SR. 1987. Spore ornamentation for North American *Phellodon* species. Mycologia 79: 334–337. http://dx.doi.org/10.2307/3807670

Banker HJ. 1906. A contribution to a revision of the North American *Hydnaceae*. Mem. Torrey Bot. Club 12: 99–124.

Banker HJ. 1913a. Type studies in the *Hydnaceae*. a. The genus *Sarcodon*. Mycologia 5: 12–17. http://dx.doi.org/10.2307/3753221

Banker HJ. 1913b. Type studies in the *Hydnaceae*. b. The genus *Phellodon*. Mycologia 5: 62–66.

Banker HJ. 1913c. Type studies in the *Hydnaceae*. c. The genus *Hydnellum*. Mycologia 5: 94–205.

Cibula WG. 1979. Fungi of the Gulf Coast I. Two new species of *Hygrophorus* section *Hygrocybe*. Mycotaxon 10: 105–115.

Cibula WG, Weber NS. 1996. *Hygrocybe andersonii* a new psammophilus *Hygrocybe* from Horn Island, a Mississippi barrier island. Mycologia 88: 514–516. http://dx.doi.org/10.2307/3760892

Coker WC. 1919. The *Hydnaceae* of North Carolina. Jour. Elisha Mitch. Sci. Soc. 34: 163–197.

Coker WC. 1926. Further notes on hydnums. Jour. Elisha Mitch. Sci. Soc. 41: 270–287.

Coker WC. 1927. New or noteworthy basidiomycetes. Jour. Elisha Mitch. Sci. Soc. 43: 129–131.

Coker WC. 1939. New or noteworthy basidiomycetes. Jour. Elisha Mitch. Sci. Soc. 55: 34–39.

Coker WC. 1942. Notes on rare hydnums. Jour. Elisha Mitch. Sci. Soc. 58: 94–97.

Coker WC, Beers AH. 1951. The stipitate hydnums of the eastern United States. Univ. N C. Press, Chapel Hill. 211 p.

Dukes GH. 2000 Mushrooms of Mississippi and other fungi and protista. Poplar Petal Pub. Co., Brandon, MS. 301 p

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes — application to identification of mycorrhizae and rusts. Mol. Ecol. 2: 113–118. http://dx.doi.org/10.1111/j.1365-294X.1993.tb00005.x

Jones TH. 1935. The *Hydnaceae* of Tennessee. Unpublished M.S. thesis, Knoxville, TN. 175 p.

Katoh K, Kuma K, Toh H, Miyata T. 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Res. 33: 511–518. http://dx.doi.org/10.1093/nar/gki198

Kornerup A, Wanscher JH. 1978. Methuen handbook of color. Sankt Jorgen Tryk Ltd, Copenhagen. 252 p.

Lickey EG, Tieken SM, Hughes KW, Petersen RH. 2007. The mushroom TWIG: A marvelous mycological menagerie in the mountains. Southeastern Nat. 1: 73–82. http://dx.doi.org/10.1656/1528-7092(2007)6(73:TMTAMM]2.0.CO;2

Posada D. 2008. jModelTest: phylogenetic model averaging. Mol. Biol. Evol. 25: 1253. http://dx.doi.org/10.1093/molbev/msn083

Ronquist F, Huelsenbeck JP. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574.

White TJ, Bruns T, Lee SB, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. 315–322, in: MA Innis et al. (eds). PCR Protocols: a guide to methods and applications. Academic Press, San Diego, CA, USA.